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(57) Abstract

The present invention provides methods and compositions for improved magnetic resonance imaging and spectroscopy. The compositions are of the general formula: C_n-L_x-G_y, wherein n is about 60 to about 1,000; L is a bifunctional linker; x is from about 0 to about 12; G is a chelator; and Y is from about 0 to about 12. Also disclosed are diagnostic compositions and methods of performing diagnostic procedures which involve administering to a warm-blooded animal a diagnostically effective amount of the compositions of the invention and then exposing the warm-blooded animal to an imaging procedure.

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COMPOSITIONS AND METHODS FOR MAGNETIC RESONANCE IMAGING, X-RAY IMAGING AND RADIOPHARMACEUTICALS

Field Of The Invention

The invention relates to magnetic resonance imaging, (MRI), x-ray imaging, and radiopharmaceuticals. More particularly the invention relates to methods and compositions for enhancing MRI, x-ray imaging and radiopharmaceuticals.

BACKGROUND OF THE INVENTION

The technique of MRI encompasses the detection of certain atomic nuclei (those possessing magnetic dipole moments) utilizing magnetic fields and radio-frequency radiation. It is similar in some respects to X-ray computed tomography ("CT") in providing a cross-sectional display of the body organ anatomy with excellent resolution of soft tissue detail. The technique of MRI is advantageously non-invasive as it avoids the use of ionizing radiation.

The hydrogen atom, having a nucleus consisting of a single unpaired proton, has the strongest magnetic dipole moment of any nucleus. Since hydrogen occurs in both water and lipids, it is abundant in the human body. Therefore, MRI is most commonly used to produce images based upon the distribution density of protons and/or the relaxation times of protons in organs and tissues. Other nuclei having a net magnetic dipole moment also exhibit a nuclear magnetic resonance phenomenon which may be used in MRI, MRS, and MRSI applications. Such nuclei include carbon-13 (six protons and seven neutrons), fluorine-19 (9 protons and 10 neutrons), sodium-23 (11 protons and 12 neutrons), and phosphorus-31 (15 protons and 16 neutrons).

While the phenomenon of NMR was discovered in 1945, it is only relatively recently that it has found application as a means of mapping the internal structure of the body as a result of the original suggestion of Lauterbur (Nature, 242, 190-191 (1973)). The fundamental lack of any known hazard associated with the level of the magnetic and radio-frequency fields that are employed renders it possible to make repeated scans on vulnerable individuals. Additionally, any scan plane can readily be selected, including transverse, coronal, and sagittal sections.

In an MRI experiment, the nuclei under study in a sample (e.g. protons, ^{19}F, etc.) are irradiated with the appropriate radio-frequency (RF) energy in a controlled gradient magnetic field. These nuclei, as they relax, subsequently emit RF energy at a sharp resonance frequency. The resonance frequency of the nuclei depends on the applied magnetic field.

According to known principles, nuclei with appropriate spin when placed in an applied magnetic field (B, expressed generally in units of gauss or Tesla (10^4 gauss)) align in the direction of the field. In the case of protons, these nuclei precess at a frequency, F, of 42.6 MHz at a field strength of 1 Tesla. At this frequency, an RF pulse of radiation will excite the nuclei and can be considered to tip the net magnetization out of the field direction, the extend of this rotation being determined by the pulse, duration and energy. After the RF pulse, the nuclei "relax" or return to equilibrium with the magnetic field, emitting radiation at the resonant frequency. The decay of the emitted radiation is characterized by two relaxation times, T_1 and T_2. T_1 is the spin-lattice relaxation time or longitudinal relaxation time, that is, the time taken by the nuclei to return to equilibrium along the direction of the externally applied magnetic field. T_2 is the spin-spin

relaxation time associated with the dephasing of the initially coherent precession of individual proton spins. These relaxation times have been established for various fluids, organs, and tissues in different species of mammals.

In MRI, scanning planes and slice thicknesses can be selected. This selection permits high quality transverse, coronal and sagittal images to be obtained directly. The absence of any moving parts in MRI equipment promotes a high reliability. It is believed that MRI has a greater potential than CT for the selective examination of tissue characteristics. The reason for this being that in CT, X-ray attenuation and coefficients alone determine image contrast, whereas at least four separate variables (T_1, T_2, proton density, and flow) may contribute to the MRI signal. For example, it has been shown (Damadian, Science, 171, 1151 (1971)) that the values of the T_1 and T_2 relaxation in tissues are generally longer by about a factor of two (2) in excised specimens of neoplastic tissue compared with the host tissue.

By reason of its sensitivity to subtle physicochemical differences between organs and/or tissues, it is believed that MRI may be capable of differentiating different tissue types and in detecting diseases which induce physicochemical changes that may not be detected by X-ray or CT which are only sensitive to differences in the electron density of tissue.

As noted above, two of the principal imaging parameters are the relaxation times, T_1 and T_2. For protons and other suitable nuclei, these relaxation times are influenced by the environment of the nuclei (e.g., viscosity, temperature, and the like). These two relaxation phenomena are essentially mechanisms whereby the initially imparted radio-frequency energy is dissipated to the surrounding

environment. The rate of this energy loss or relaxation can be influenced by certain other nuclei which are paramagnetic. Chemical compounds incorporating these paramagnetic nuclei may substantially alter the T_1 and T_2 values for nearby nuclei having a magnetic dipole moment. The extent of the paramagnetic effect of the given chemical compound is a function of the environment within which it finds itself.

A need continues to exist for contrast agents that will enhance images of body organs and tissues. Such agents are disclosed and claimed in this document.

SUMMARY OF THE INVENTION

The present invention provides methods and compositions for improved magnetic resonance imaging, spectroscopy, and radiopharmaceuticals. The compositions are of the general formula:

$$C_n\ L_x\ G_Y$$

wherein n is about 60-about 1000; L is a bifunctional linker; x is from about 0 to about 12; G is a chelator; and Y is from about 0 to about 12. Also provided are compositions of the general formula:

$$C_n\ L_x\ G_y\ M_z$$

wherein n is about 60 to about 1000; L is a bifunctional linker; x is from 0 to about 12; G is a chelator; Y is from 0 to about 12; M is a paramagnetic ion, radioactive

metal ion or x-ray absorbing ion; and z is from about 1 to about 12.

Also disclosed are diagnostic compositions and methods of performing diagnostic procedures which involve administering to a warm-blooded animal (including humans) a diagnostically effective amount of the compositions of the invention and then exposing the warm-blooded animal to an imaging procedure.

DETAILED DESCRIPTION OF THE INVENTION

Stable C_{60} closed carbon shells have recently been isolated from vaporized graphite. The highly stable C_{60} compound is marked by an icosahedral-cage structure, a polygon with 60 equivalent vertices, 32 faces, 12 of which are pentagonal and 20 hexagonal. The icosahedral structure is typified by a soccer ball. The C_{60} structure has been given the name "buckminsterfullerene" due to its similarity to the geodesic domes of Buckminster Fuller. The class of closed cage, carbon clusters is commonly referred to as "fullerenes."

C_{60} is the prototypical fullerene. A number of methods for the formation and purification of C_{60} have been developed and are known in the art. Generally, pure graphitic carbon is vaporized in an inert atmosphere, and C_{60} is extracted from the deposited soot with benzene, toluene, carbon disulfide, or carbon tetrachloride. The extract consists primarily of C_{60} and C_{70}. Other stable low molecular weight fullerenes have also been identified, such as C_{24}, C_{28}, C_{32}, and C_{50}. The existence of high

molecular weight fullerenes, such as C_{240} and C_{540}, is theoretically predicted.

C_{60} exhibits extended aromaticity and has been found to be a sensitizer. Chemical modification of the C_{60} structure is necessary to prepare compositions suitable for in vivo applications. Hydrogenation of fullerenes is achieved using known techniques, such as catalytic hydrogenation or dissolving metal reduction. The partially hydrogenated compounds, $C_{60}H_{36}$ and $C_{60}H_{18}$, are readily formed. Complete hydrogenation to $C_{60}H_{60}$ by catalytic hydrogenation may be accomplished using higher pressures of H_2 and variation of catalyst. In addition to hydrogenated species, fluorinated, heterocyclic, and other functionalized derivatives of the C_{60} structure have been prepared.

By vaporizing graphite impregnated with a suitable paramagnetic metal species, it is possible to produce fullerene cages containing a paramagnetic metal species. The term paramagnetic metal species as used herein includes within its scope both paramagnetic atoms and ions. Also, the presence of a paramagnetic metal species may enhance MRI, MRS, and MRSI. It is also believed that incorporating a paramagnetic metal species into the center of the fullerene cage will increase the dipole moment of the entire cage. This may render the cluster water soluble and reduce in vivo toxicity of the paramagnetic metal species.

Fullerenes, in particular C_{60}, have much higher reactivity than might be expected based on their inherent stability resulting from an aromatic-type structure consisting of twenty 6-membered rings fused to twelve 5-membered rings. Buckyball (C_{60}) is insoluble in water and most organic solvents except for benzene (1.44 mg/mL),

toluene (2.15 mg/mL), and carbon disulfide (5.16 mg/mL); reduced fullerenes are highly soluble in THF, however. Examples of some types of reactions that C_{60} undergoes are: nucleophilic and electrophilic and radical additions, Friedel-Crafts, Diels-Alder, electrocyclic, hydroboration, cycloaddition, electrophilic aromatic substitution, reductive alkylation, and halogenation. In addition, C_{60} has been shown to react with organometallic transition metal complexes (Balch, A. J., *et al. Inorg. Chem.* **1991**, 30, 3980 and Fagan, P. J., *et al. Acc. Chem. Res.* **1992**, 25, 134). It is important to note that upon functionalization of C_{60}, in many cases, the resulting C_{60} derivatives are water soluble or can be derivatized to be such; moreover, by boiling an aqueous solution of γ-cyclodextrin with a solid mixture of C_{60} and C_{70}, researchers have been able to extract C_{60} from the mixture into the aqueous solution (Andersson, T., *et al.*).

Treatment of buckyball (C_{60}) or higher homologues (C_n,n>60) with chelators for paramagnetic metal ions (e.g., Gd(III) or Mn(II)) bearing pendant nucleophilic groups should afford $\{C_{60}(\text{chelator})_x\}$, where X = 1-12 (referred to as fuzzyball). Appropriate nucleophilic groups include amines, alkoxides, thiolates, and carbanions derived from carbonyl compounds. In an alternative formulation, attachment of chelators to C_{60} could be effected through a linker group. Functionalization of C_{60} with a linker group (e.g., sodium diethyl malonate, followed by decarboxylation and esterification) and subsequent reaction with a chelator bearing a suitable pendant nucleophilic group (e.g., primary amine) also leads to fuzzyball. Reaction of fuzzyball with a paramagnetic metal, such as those

mentioned in this document, affords a compound with a potentially large (6-14) number of paramagnetic metal ions. High relaxivity results not only because of the large number of paramagnetic metal ions, but also because of the exceptionally slow tumbling of fuzzyball. Minimal osmolarity (compared to an equivalent concentration of similar free chelators) results from combination of free complexes into a single particle in solution. Potential MRI applications include use as a contrast agent for extracellular fluid or the blood pool, or for attachment to targeting groups (especially monoclonal antibodies). In the latter case exceptionally high relaxivity is critical to successful contrast enhancement at a practical loading of the MAb with the contrast agent. Fuzzyball with Gd(III) might also find application as a non-conventional X-ray contrast agent.

In general, paramagnetic ions of elements with an atomic number of 21 to 29, 42 to 44, and 58 to 70 have been found effective as MRI contrasting agents. Examples of suitable paramagnetic ions for use with the invention include chromium(III), manganese(II), manganese(III), iron(III), iron(II), cobalt(II), nickel(II), copper(II), praseodymium(III), neodymium(III), samarium(III) and ytterbium(III). Due to their very strong magnetic moments, gadolinium(III), terbium(III), dysprosium(III), holmium(III) and erbium(III) are preferred. Gadolinium(III) ions have been particularly preferred as MRI contrasting agents.

Examples of suitable bifunctional linkers for use with the invention include ethylenediamine, ethanolamine, B-alanine, 1,4-diaminobutane, allyl amine,

mercaptoethylamine, propylenediamine, mercaptoethanol, 3-mercaptoprionic acid, allyl mercaptan, 1,2-propanedithiol, 1,2-ethanedithiol, allyl magnesium bromide, phenyl magnesium bromide, phenyl lithium, and diethylmalonate. Generally nucleophilic groups work well. Examples of nucleophilic groups include amines, amides, alcohols, phenols, thiols and hydrazines. The more linkers used generally increases the chances of getting a larger number of chelators attached, and therefore a greater number of metals bound. With radiopharmaceuticals, however, only one bound metal is generally necessary.

Linkers are chosen for their reactivity with the carbon cage. One site of the linker generally reacts with the carbon cage and another site of the linker generally reacts with the site of the chelator.

Examples of suitable chelators for use with the invention include diethylenetriamine pentaacetic acid (DTPA), ethylene diamine tetraacetic acid (EDTA), 1, 4, 7, 10-tetraazacyclododecane tetraacetic acid (DOTA), mercaptoacetylglycyl glycylglycine (MAG3), 1, 4, 8, 11 tetraaza-cyclotetradecane (cyclam), N, N'-bis(O)-hydroxybenzyl) ethylene diamine N,N'-diacetic acid (HBED), and 2, 2, 9, 9-tetramethyl-4, 7-diaza-1, 10-decanedithiol. The chelator should be capable of binding a desired metal.

Biomolecule refers to all natural and synthetic molecules that play a role in biological systems. Biomolecules include hormones, amino acids, peptides, peptidomimetics, proteins, deoxyribonucleic acid (DNA) ribonucleic acid (RNA), lipids, albumins, polyclonal antibodies, receptor molecules, receptor binding molecules, monoclonal antibodies and aptamers. Specific examples of biomolecules include insulins, prostaglandins, growth factors, liposomes and nucleic acid probes.

Examples of synthetic polymers include polylysine, arborols, dendrimers, and cyclodextrins. The advantages of using biomolecules includes enhanced tissue targeting through specificity and delivery. The biomolecule can be attached to a variety of places on the molecules of the invention. Coupling of the chelating moieties to biomolecules can be accomplished by several known methods (e.g., Krejcarek and Tucker Biochem. Biophys. Res. Comm., **30**, 581 (1977); Hnatowich, et al. Science, **220**, 613 (1983). For example, a reactive moiety present on the chelating moiety, fuzzyball or linker is coupled with a second reactive group located on the biomolecule. Typically, a nucleophilic group is reacted with an electrophilic group to form a covalent bond between the biomolecule and the chelate. Examples of nucleophilic groups include amines, anilines, alcohols, phenols, thiols and hydrazines. Electrophilic group examples include halides, disulfides, epoxides, maleimides, acid chlorides, anhydrides, mixed anhydrides, activated esters, imidates, isocyanates and isothiocyanates.

In addition to their utility in magnetic resonance imaging procedures, the compositions of the invention can also be employed for delivery of either radiopharmaceuticals or heavy metals for x-ray contrast into the body. For use in diagnostic and therapeutic radiopharmaceuticals the complexed metal ion must be radioactive. Radioisotopes of the elements technetium, rhenium, indium, gallium, copper, yttrium, samarium and holmium are suitable. For use as x-ray contrast applications the complexed metal ion must be able to absorb adequate amounts of the x-rays. These metal ions are generally referred to as radioopaque. Suitable

elements for use as the radioopaque metal ion include lead, bismuth, gadolinium, dysprosium and praseodymium.

Advantageously, the compositions may further contain physiologically acceptable non-toxic cations in the form of a gluconate, chloride or other suitable organic or inorganic salts, including suitable soluble complexes with a chelate/ligand to enhance safety. Examples of suitable non-toxic cations include sodium ions, calcium ions, magnesium ions, copper ions, zinc ions, and mixtures thereof.

The compositions of the invention can be formulated into diagnostic compositions for enteral or parental administration. These compositions contain an effective amount of the complex along with conventional pharmaceutical carriers and excipients appropriate for the type of administration contemplated. For example, parenteral formulations advantageously contain a sterile aqueous solution or suspension of from about 0.05 to about 1.0 M of an ion complex. Parenteral compositions may be injected directly or mixed with a large volume parenteral composition for systemic administration. Preferred parenteral formulations have a complex concentration of about 0.1M to about 0.5M. Such solutions also may contain pharmaceutically acceptable buffers and, optionally, electrolytes such as sodium chloride. The compositions may advantageously contain a slight excess (e.g., from about 0.01 to about 15.0 mole % excess) of a complexing agent or its complex with a physiologically acceptable, non-toxic cation. Such physiologically acceptable, non-toxic cations include calcium ions, magnesium ions, copper ions, zinc ions, salts of n-methylglucamine and diethanolamine, and the like. Generally, calcium ions are preferred.

Formulations for enteral administration may vary widely, as is well-known in the art. In general, such formulations are liquids which include an effective amount of the paramagnetic ion complex in aqueous solution or suspension. Such enteral compositions may optionally include buffers, surfactants, thixotropic agents, and the like. Compositions for oral administration may also contain flavoring agents and other ingredients for enhancing their organoleptic qualities.

The diagnostic compositions are administered in doses effective to achieve the desired enhancement of the NMR image. Such doses may vary widely, depending upon the particular ion complex employed, the organs or tissues which are the subject of the imaging procedure, the imaging procedure, the imaging equipment being used, and the like. In general, parenteral dosages will range from about 0.001 to about 1.0 mMol of ion complex per kg of patient body weight. Preferred parenteral dosages range from about 0.01 to about 0.5mMol of ion complex per kg of patient body weight. Enteral dosages generally range from about 0.5 to about 100 mMol, preferably from about 1.0 to about 10 mMol, preferably from about 1.0 to about 20.0 mMol of ion complex per kg of patient body weight.

The diagnostic compositions of the invention are used in the conventional manner. The compositions may be administered to a patient, typically a warm-blooded animal, either systemically or locally to the organ or tissue to be imaged, and the patient then subjected to the imaging procedure. Protocols for imaging and instrument procedures are found in texts such as Stark, D.D.; Bradley, W.G. *Magnetic Resonance Imaging*; Mosby Year Book: St. Louis, MO, 1992.

Radiopharmaceutical imaging procedures are found in Fred A. Mettler, Jr., M.D., M.P.H., Milton J. Guiberteau, M.D., Essentials of Nuclear Medicine Imaging, Grune and Stratton, Inc., New York, NY 1983) and E. Edmund Kim, M.S., M.D. and Thomas P. Haynie, M.D., (MacMillan Publishing Co. Inc., New York, NY 1987).

XRCM imaging procedures are found in Albert A. Moss, M.D., Gordon Gamsu, M.D., and Harry K. Genant, M.D., Computed Tomography of the Body, (W.B. Saunders Company, Philadelphia, Pennsylvania 1992) and M. Sovak, Editor, Radiocontrast Agents, (Springer-Verlag, Berlin 1984).

The following examples illustrate the specific embodiments of the invention described in this document. As would be apparant to skilled artisans, various changes and modifications are possible and are contemplated within the scope of the invention described.

EXAMPLES

Example 1

Preparation of C_{60}(ethylenediamine)$_{10}$:

Ethylenediamine is dried via azeotropic distillation with toluene. To 100 mL of this ethylenediamine is added 0.50g of C_{60}. The reaction is allowed to stir overnight under argon. The product is extracted with

tetrahydrofuran (THF). Following the removal of the THF, a beige solid remains.

Example 2

Preparation of C_{60}(ethylenediamine)$_{10}$(DTPA)$_{10}$:

To 1.0 g of the C_{60}(ethanolamine)$_{10}$ prepared in substantial accordance with the teachings of Example 1 is added a 10 molar equivalent of diethylenetriaminepentaacetic acid (DTPA) bis anhydride in 50 mL of DMF. The reaction mixture is heated to 40-50 C. The anhydride is cleaved by acid hydrolysis. After removal of the solvent under reduced pressure, a solid results.

Example 3

Preparation of C_{60}(ethylenediamine)$_{10}$(DTPA)$_{10}$(Gd)$_{10}$:

To the solid product obtained in substantial accordance with the teaching of Example 2 is added an excess (12 molar equivalents) of $GdCl_3$ in dimethylacetamide. Following purification on an ion-exchange resin, the major product obtained is of the formula C_{60}(ethylenediamine)$_{10}$(DTPA)$_{10}$(Gd)$_{10}$.

Example 4

Preparation of C_{60}(1,4-diaminobutane)$_6$:

50 mL of 1,4-diaminobutane are purified by refluxing over Na spheres followed by distillation under reduced pressure. To this liquid is added 0.50g of C_{60}. This

reaction mixture is stirred overnight at room temperature under argon. The excess amine is removed via steam distillation. The resulting gold syrup-like product is extracted with chloroform and the chloroform-soluble layer evaporated to yield a brown solid.

Example 5

Preparation of C_{60}(1,4-diaminobutane)$_6$(DOTA)$_6$:

The free acid form of 1,4,7,10-tetraazycyclo dodecane tetraacetic acid (DOTA, 3.2g, 8.0 mmol) and triethylamine (3.2g, 32 mmol) are dissolved in 50 mL of dry dimethylsulfoxide (DMSO) by gentle warming. The homogeneous solution is cooled to room temperature and isobutyl chloroformate (1.1g, 8.0 mmol) is added dropwise, followed by the addition of 1.4 g of the C_{60}(1,4-diaminobutane)$_6$ adduct obtained in substantial accordance with the teaching of Example 4. The mixture is stirred for several hours and the DMSO is distilled off under vacuum. The residue is purified on an anion-exchange resin. The clean fractions are combined and evaporated to afford the desired product.

Example 6

Preparation of C_{60}(1,4-diaminobutane)$_6$(DOTA)$_6$Dy$_6$:

Six molar equivalents of $Dy_2O_3(PPh_3)_2$ are suspended in 25 mL of dry CH_2Cl_2 and the solution purged with argon. To this suspension is added 1 equivalent of the C_{60}(1,4-diaminobutane)$_6$(DOTA)$_6$ product obtained in substantial accordance with the teaching of example 5. The reaction mixture is stirred at room temperature for 8

hours and the solvent removed by rotary evaporation. Following chromatographic purification the desired product is isolated.

Example 7

Preparation of C_{60}(1,4-diaminobutane)$_2$

The C_{60}(1,4-diaminobutane)$_2$ adduct is prepared in substantial accordance with the teaching of Example 4, except that only 2 molar equivalent of the amine is reacted with the C_{60}.

Example 8

Preparation of C_{60}(1,4-diaminobutane)$_2$(MAG3)(epsilon-t-Boc octreotide):

A mixture of the C_{60}(1,4-diaminobutane) adduct (1.4g, 1.0mmol) and s-tetrahydropyranyl-mercaptoacetylglycylglycylglycine-N-hydroxysuccinimide active ester (MAG3, 3.1g, 1.0 mmol) in 25 mL of DMSO is stirred for 2 hours at room temperature. The remaining primary amine on the C_{60} moiety is reacted with N, N^1-disuccinimidyl carbonate (256 mg, 1.0 mmol) followed by the addition of epsilon-t-boc-octreotide (1.0 mmol). The solution is stirred for 2 hours at room temperature. The DMSO is removed at reduced pressure. The residue is purified using reversed-phase chromatography to afford the desired product.

Example 9

Preparation of C_{60}(1,4-diaminobutane)$_2$(MAG3)(octreotide) ^{186}Re:

A mixture of the C_{60}(1,4-diaminobutane)(MAG3)(epsilon-t-Boc octreotide) ligand (1.0 mg), stannous chloride (0.5 mg, 2.6×10^{-6} mol), sodium citrate (28.8 mg, 1.5×10^{-4} mol), and ^{186}Re perrhenate (4.25 μg, 2.3×10^{-8} mol) in 100 μL of water and 100 μL of acetonitrile is heated at 50 C for 1 hour. A volume of 0.30 mL of 1 N NaOH is added. The rhenium complex is purified via C-18 chromatography using water/methanol as the eluant to wash off impurities. The desired complex is eluted with acetonitrile then evaporated to dryness to afford the C_{60}(1,4-diaminobutane)(MAG3)(octreotide)^{186}Re complex.

Although the invention has been described with respect to specific modifications, the details thereof are not to be construed as limitations, for it will be apparent that various equivalents, changes and modifications may be resorted to without departing from the spirit and scope thereof, and it is understood that such equivalent embodiments are to be included therein.

What is claimed is:

1. A composition comprising the formula:

$$C_nL_xG_y$$

wherein n is about 60 to about 1000; L is a bifunctional linker; x is from about 0 to about 12; G is a chelator; and Y is from about 0 to about 12, provided x or y is at least 1.

2. The composition of claim 1 wherein the bifunctional linker is selected from the group consisting of ethylenediamine, ethanolamine, B-alanine, 1,4-diaminobutane, allyl amine, mercaptoethylamine, propylenediamine, mercaptoethanol, 3-mercaptopropionate acid, allyl mercaptan, 1,2-propanedithiol, 1,2-ethanedithiol, allyl magnesium bromide, phenyl magnesium bromide, phenyl lithium, and diethylmalonate.

3. The composition of claim 2 wherein the chelator is selected from the group consisting of diethylenetriamine pentaacetic acid (DTPA), ethylene diamine tetraacetic acid (EDTA), 1, 4, 7, 10-tetraazacyclododecane tetraacetic acid (DOTA), mercaptoacetylglycyl glycylglycine (MAG3), N, N'-bis(O)-hydroxybenzyl) ethylene diamine N,N'-diacetic acid (HBED), and 2, 2, 9, 9-tetramethyl-4, 7-diaza-1, 10-decanedithiol.

4. The composition of claim 3 further comprising a metal ion of chromium(III), manganese(II), manganese(III), iron(III), iron(II), cobalt(II), nickel(II), copper(II), praseodymium(III),

neodymium(III), samarium(III), ytterbium(III), gadolinium(III), terbium(III), dysprosium(III), holmium(III), erbium(III), technetium, rhenium, indium, gallium, copper, yttrium, samarium, holmium, lead, bismuth, gadolinium, dysprosium and praseodymium.

5. The composition of claim 4 further comprising a biomolecule selected from the group consisting of hormones, amino acids, peptides, peptidomimetics, proteins, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), lipids, albumins, polyclonal antibodies, receptor molecules, receptor binding molecules, monoclonal antibodies, and aptamers.

6. A composition comprising the formula $C_nL_xG_y$ wherein n is 60, L is ethylendiamine, x is 10, G is DTPA, and y is 10.

7. A composition comprising the formula $C_nL_xG_y$ wherein n is 60, L is ethylenediamine, x is 10, G is EDTA, and y is 10.

8. The composition of claim 7 further comprising gadolinium.

9. The composition of claim 1 wherein n is 60, L is 1,4-diaminobutane, x is 6, G is DOTA, and y is 6.

10. The composition of claim 4 wherein n is 60, L is 1,4-diaminobutane, x is 6, G is cyclam, y is 6, and the metal ion is dysprosium.

11. A composition of claim 5 wherein n is 60, L is 1,4-diaminobutane, x is 2, G is MAG3, and the biomolecule is octreotide.

12. A method of imaging which comprises administering a composition of the formula:

$$C_nX_xG_yM$$

wherein n is about 60 to about 1000; L is a bifunctional linker; x is from 0 to about 12; G is a chelator; Y is from 0 to about 12; and M is a metal ion.

13. The method of claim 12 wherein the bifunctional linker is selected from the group consisting of ethylenediamine, ethanolamine, B-alanine, 1,4-diaminobutane, allyl amine, mercaptoethylamine, propylenediamine, mercaptoethanol, 3-mercaptoproprionate acid, allyl mercaptan, 1,2-propanedithiol, 1,2-ethanedithiol, allyl magnesium bromide, phenyl magnesium bromide, phenyl lithium, and diethylmalonate.

14. The method of claim 13 wherein the chelator is selected from the group consisting of diethylenetriamine pentaacetic acid (DTPA), ethylene diamine tetraacetic acid (EDTA), 1, 4, 7, 10-tetraazacyclododecane tetraacetic acid (DOTA), mercaptoacetylglycyl glycylglycine (MAG3), N, N'-bis(O)-hydroxybenzyl) ethylene diamine N,N'-diacetic acid (HBED), and 2, 2, 9, 9-tetramethyl-4, 7-diaza-1, 10-decanedithiol.

15. The method of claim 14 wherein the metal ion is chromium(III), manganese(II), manganese(III), iron(III),

iron(II), cobalt(II), nickel(II), copper(II), praseodymium(III), neodymium(III), samarium(III), ytterbium(III), gadolinium(III), terbium(III), dysprosium(III), holmium(III), erbium(III), technetium, rhenium, indium, gallium, copper, yttrium, samarium, holmium, lead, bismuth, gadolinium, dysprosium, or praseodymium.

16. The method of claim 15 wherein the composition further comprises a biomolecule

17. The method of claim 16 wherein the metal ion is gadolinium.

18. The method of claim 15 wherein n is 60, L is 1,4-diaminobutane, x is 6, G is cyclam, y is 6, and the metal ion is dysprosium.

19. The method of claim 16 wherein the composition further comprises a biomolecule selected from the group consisting of hormones, amino acids, peptides, peptidomimetics, proteins, deoxyribonucleic acid (DNA) ribonucleic acid (RNA), lipids, albumins, polyclonal antibodies, receptor molecules, receptor binding molecules, monoclonal antibodies, and aptamers.

20. The method of claim 19 wherein the biomolecule is octreotide.

INTERNATIONAL SEARCH REPORT

Int. ..tional application No.
PCT/US94/10999

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61B 5/055; C01F 17/00; C01B 31/00; C07C 19/08
US CL :424/9; 423/263, 445; 128/653.4, 654; 436/173; 534/15; 570/130
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/9; 423/263, 445; 128/653.4, 654; 436/173; 534/15; 570/130

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE, APS
search terms: buckyball, fullerene, chelate

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	US, A, 5,177,248 (Chiang et al.) 05 January 1993, see column 1, lines 54-61 and abstract.	1-4, 6-10, 12-15 and 18 ---------- 1-20
X	WO, A, 93/15,768 (Watson, et al.) 19 August 1993, see pages 7-9 and 16.	1-20
A	US, A, 5,248,498 (Neumann et al.) 28 September 1993, see abstract.	1-20
A	US, A, 5,223,479 (McCauley, Jr. et al.) 29 June 1993, see abstract.	1-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 18 NOVEMBER 1994	Date of mailing of the international search report 10 JAN 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer GARY E. HOLLINDEN Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

Inte...tional application No.
PCT/US94/10999

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Inter...ional application No.
PCT/US94/10999

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

I. Claims 1-4, 6-10, 12-15, and 18, drawn to a compound containing a fullerene moiety and either a linking or a chelating moiety (or both) and a method of using said compound for magnetic resonance imaging (MRI).

II. Claims 5, 11, 16, 17, 19, and 20, drawn to a compound containing a fullerene moiety and either a linking or a chelating moiety (or both) and further containing a biomolecule and a method of using said compound for magnetic resonance imaging (MRI).

The inventions listed as Groups I and II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Clearly, a reference which would anticipate Group I would not necessarily anticipate or even make obvious the invention(s) of Group II. Further, the searches of the inventions are not co-extensive, particularly with regard to the literature search required. One skilled in the art could readily practice the invention of Group I without practicing the invention(s) of Group II. Since the two groups of compounds represent independent classes of compounds with very distinct scope and since the inclusion of the biomolecule of group II is clearly a new special technical feature not found in Group I, a lack of unity requirement is appropriate.